

IV. Discussion

The present experiments were based on the following set of questions:

1. Does the smoke-treatment method which we developed represent a reproducible biological test?
2. Which of the obtained findings are attributable to the smoke-treatment of the animals and can we establish a dose-effect correlation?
3. Are quantitative differences ascertainable between the results from the individual test groups?
4. Can we draw conclusions from the combined treatments with smoke and various carcinogenic substances which would point to a co-carcinogenic effect, respectively an over-additive - in the sense of a co-carcinogenic - effect?
5. Evaluation of the remaining obtained findings which do not show a correlation to the treatment.

I. Smoke-treatment experiments of the same extent have so far not been conducted so that a basis for comparisons was not available. The vastly standardized biological tests in the area of the skin and the subcutaneous tissue permit solely the evaluation of the effect of the particle-phase of the smoke, mostly only through the inclusion of a solvent. Aging processes of the smoke as well as possible alterations of the biological effect through lack of the gas-phase are as much of a handicap in the epicutan-test as the various reactions of the skin, respectively the respiration epithelium, depending on anatomical and functional data. The same holds true for a subcutaneous application of the smoke-condensate.

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A decisive criterion for the particularly good suitability of the testing method is also the fact that it allows for information on the effect of the total smoke, whereby

the freshly offered smoke comes to an effect in a quality which is equivalent to the smoke inhaled by the smoker. This decisive advantage (in the skin-test, it is the compensated particle-phase of the smoke, mostly days old, which is being tested) gives a greater validity to the information from the inhalation test, as well as the fact, that the smoke becomes primarily effective on the same organs as in the smoker, and arrives in the organism through the same ways as it is the case in the smoker. The reproducibility of our test shows that the methodology developed by us represents a useful biological test. It was in the present test, despite the use of another animal breed, not only possible to obtain vastly similar findings as in the first test (DONTENWILL 1970). It became evident that in two groups (groups 5 and I2) treated in approximately the same way, vastly similar findings appeared in the area of the larynx. The simultaneous asbestos treatment of group I2 is thereby negligible because of its dose and effect which were too small, as the comparison to group I3, treated solely with asbestos demonstrates.

2. As chief effects of the smoke-components must be assessed the changes in the larynx, the hemoglobin and in the numbers of erythrocytes, the change in the life-expectancy and changes in the body-weight as well as the increase of certain changes in the lung like "smoke-cells".

In most of the animals, smoke-treated over a longer period of time (approximately 1 year), changes are provable on the locations in the respiratory tract on which the strongest concentration of the smoke-components in the sense of a deposit takes place. While in the larynx of an overwhelming number of the 960 control animals and animals treated with fresh air, no changes appeared, or at least only minor metaplastic changes of the epithelium of stage I and in one animal of stage 2, we found in animals, treated over a longer period of time with the total smoke, considerable epithelium changes of the stages 3 to 5 and carcinomas (stage 6) in large numbers. In studying the charts it becomes easily evident that the differences between the controls and

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the treated animals must be termed unequivocal. This fact alone makes a comparative evaluation possible. It is facilitated even more, and the validity of the results is increased, when we compare the individual stages, according to the experiences in experimental cancer research with evaluation of the histological structure, to the change of the skin. The stages 3 and 4 in their significance could be most closely compared to papillomas of the mouse-skin. Stage 5 should be included in the group precanceroses "in the narrower meaning" which the term "pseudoepitheliomatous leukoplakia" was intended to express (see Atlas of the Armed Forces Institute). Stage 5 would perhaps be evaluated differently in a part of its cases by individual researchers. In the schematic representation we tried to characterize the stages morphologically in order to stress, for instance, the difference between stage 5 and the carcinoma. Only those changes were termed beginning carcinomas (stage 6) (early invasive carcinoma) which showed an infiltrative growing process. The frequency of the stages 5 and 6 is best suitable, in our opinion, for a comparison of the biological effect between two different test groups. When we evaluate the frequency of stage 6 alone, that is, of the carcinomas, the comparison can be established in the simplest way.

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The observation of the temporal succession of the individual stages shows that, whenever the biological effect is the least, the stages 5 and 6 appear more rarely, the preliminary stages 2, 3 and 4 however, are observed more frequently. In the individual stages, particularly in 3 - 6, it is not only a case of gradual differences in the dignity, but also of epithelium changes developing in temporal sequence, in which dose and time factors as well as factors of the biological effectiveness determine decisively, which degrees of epithelium changes will appear after a certain treatment.

Of the numerous biochemical and hematological findings, the changes of the hemoglobin and the erythrocytes are of particular interest. They are, as we had reported already earlier (DONTENWILL 1970), probably contingent on the chronic, high-dosed CO-intoxication which, through the test arrangement in the smoke-treatment, is multiply higher than in

the smoking of man. The leukocyte reduction, traceable in individual groups, we attempted to explain through the reduction in weight. Surprisingly, no changes of the lipoids occurred, even though the hamster, as a literature survey demonstrates, reacts with cholesterol feeding just as strongly as other test animals. Since the present smoke-treatment experiments were conducted on large numbers of animals and at different times, these findings deserve a greater significance.

Consequences of the chronic smoke-treatment are, besides a reduced consumption of food, the distinct reduction of the body-weight increase. The longer survival period of the untreated controls in contrast to the "fresh air animals" shows as well as the evaluation of the testicle changes that the remaining in the tubes of the smoke-treatment apparatus represents a factor which has a varying effect on the individual measured values (e.g. survival period) and shows the strongest reaction in the area of the testicle weights. The findings, obtained in the comparison of the animals treated with fresh air and the untreated controls, show, how important it is, precisely in smoke-treatment testing, to have a "placebo group" which allows in the calculation of the results to recognize the influence of the "test arrangement".

The opinion, postulated by us earlier, that the "adenomatoid lesions" are cases of preliminary stages of carcinomas (CHEVALIER and DONTENWILL 1971), will not be maintained by us any longer. Apparently, these are instances of changes which can be triggered by various factors, but which are not comparable to preliminary stages of tumors.

We can say with certainty that the term "smoke cells" is not conclusive since these changes are also found in animals not treated with smoke. They must probably, like the "adenomatoid lesions", be regarded as unspecific reactions of the lung, since smoke-cells were found much more frequently in animals smoke-treated over a longer period of time.

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We gave particular attention to the appearance of emphysema. It was possible through the applied fixation method to compare quantitatively the changes in the lung. The diagnose emphysema was microscopically determined only relatively rarely and not at all unequivocally more frequently in the smoke-exposed animals. Therefore we carried out quantitative measurements with two different methods in individual test groups, with the question whether lung changes which can be termed emphysema will occur more frequently after chronic smoke-treatment. This was not the case, even though it is possible to produce a lung emphysema in the hamster through certain methods. This raises the question whether the total dosis reaching the lung is not high enough, or whether the hamster is not inclined in the same way to the formation of emphysemas as are other breeds of animals or man.

The provable dose-effect-correlation is another factor which must be assessed as an indication of the usefulness of the method. The increase in the frequency of the changes and the increase of their degree of severity in the larynx in the groups 4 and 5 shows that dose-effect-correlations are existing. The fact, that with the highest dose (group 6) a further increase was no longer provable, may have the same reasons, as we had also assumed them in our 4th Report on the Drop-Testing of Mice (DONTENWILL and Assoc. 1972). It is possible that an over-dosing, through higher toxicity and through a stronger deposit of smoke-components per unit of area which is no longer effective, will not show any more increase in the biological effectiveness. We were able to show unequivocally in our tests on the skins of mice (DONTENWILL and ASSOC. 1972) that a too high concentration of the tobacco-smoke condensate solutions (per unit of area) will no longer lead to an increase of the biological effect in the region of the treated area.

3. The present experiment is concerned as a chronic toxicity examination with the effect of the total smoke, respectively of certain smoke-components of very varied cigarettes, the smoke of which has been altered in quantity and quality through filter

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or additives, or whose manufacture from sheets has led to an alteration of the smoke-substances.

Of particular interest are therefore those test results which show an influence on the biological effect through an alteration of the product. Since the present experiment with the large number of animals and the initially mentioned factors has a relatively high validity of evidence, it appears important to point out that the risk reduction, in the sense of a reduced biological effect, which was evident in the Drop-Tests (DONTENWILL, 1970 and DONTENWILL and Assoc. 1972) can also be proven in the same way in the inhalation test. The smoke of a same number of cigarettes was offered to the animals for the purpose of a comparison. The biological effect in the area of the larynx was very varied, particularly in the evaluation of the severe epithelium changes (stage 5 and 6) or the carcinomas alone (stage 6).

Inhalation of the gas-phase components alone led, similarly as in the animals treated only with fresh air, to metaplastic changes of the larynx of stage I only in 9 of 160 animals. - The strongest reduction of the biological effect was shown by cigarettes with acetate-filter and Dico-filter, the used sheet-cigarettes with and without NaNO_3 - additive as well as by the cigarettes with the black mixture. According to these findings distinct ways of a risk reduction result solely from a reduction or qualitative alteration of the offered smoke-components with the use of filter in the sense of a dose-effect relation. These findings confirm observations by WYNDER and Assoc. (1970) and by BROSS (1968) in epidemiological examinations on smokers who prefer filter cigarettes, and by AULZBACH and Assoc. (1970) in experiments on dogs. But the tests also show that the already previously mentioned idea of an increased nitrosamine formation in cigarettes with NaNO_3 -admixture is not at all proven yet.

The reduction of the biological effect of smoke on the larynx, recognizable in the comparison between the individual groups with the same dosage (e.g. between group 5 = 2 x 30 cigarettes of the standard mixture and group 14 = 2 x 30 cigarettes of the

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black mixture) is once again discussed in chart I34.

4. Particularly unequivocal is also the difference between the animals pre-treated with DEMA and the animals treated only with smoke. It becomes evident here that the present methodology is also suitable for the interpretation of a syncarcinogenic or cocarcinogenic effect. If it were only a case of a syncarcinogenic effect, the sums of the effects of DEMA alone = group 2 and of smoke alone = group 5 should add up, and the summary effect should become evident in group I. The frequency of the stages 5 and 6 in group I is higher than the frequency of these stages in the groups 2 and 5 together, which must be interpreted as an over-additive effect. How far a cocarcinogenic effect can be drawn upon as an explanation remains open.

The tests show unequivocally, in our opinion, that the smoke-components attack in the same places as the strongly cancerogenically effective hydrocarbon DEMA, as the schematic graph 66 shows. The conclusion could be drawn from this finding that the hydrocarbons of the smoke constitute an important factor in the triggering of the epithelial changes on the larynx, and that the effect of the hydrocarbons is strengthened through cocarcinogenic factors of the smoke in the same place. That the effect is to a considerable extent a cocarcinogenic effect is confirmed by the tests of WENDER and HOFFMANN (1961), BOCK (1968, 1969), VAN DUUREN (1969), VAN DUUREN and Assoc. (1966 and 1971) and others, as well as by our own observations on the skins of mice (DONTENWILL and Assoc. 1972) which show that cancerogens contained in smoke are potentiated through cocarcinogenic factors.

The combined tests with asbestos and smoke did not yield a result since the applied asbestos quantity was apparently too small.

Interesting are the findings which were obtained from those groups treated simultaneously with smoke and diethyl-nitrosamin. We purposely did not conduct an initial treatment because it leads to too great a loss in animals through the formation of tracheal papillomas. We already pointed that out in 1964 (DONTENWILL). These former observations

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have now been confirmed by the experiments of WEHNER and Assoc. (1971) who proved, upon initial treatment with nitrosamins, an extraordinarily high mortality through papilloma formation in the trachea.

The nitrosamin treatment did not increase the frequency of the larynx changes. An explanation for that is extraordinarily difficult. When we consider the hitherto existing test results with combinations of nitrosamins and hydrocarbons, we see in a test by KOCH-LIGETTI and Assoc. (1963) in the combination of both carcinogens no increase in the tumors found. In a report by CEBALLOS and HERRANZ (1970) on the other hand, the carcinogenic effect of methylcholanthrene was reduced through an additional nitrosamin treatment.

From the present experiment we can recognize only one thing unequivocally: additional nitrosamin treatment does not produce an increase in the larynx changes produced by smoke. We were able to establish unequivocally that with nitrosamin treatment papillary epithelium growths are produced in places, as they occur in the same manner with nitrosamin treatment alone, and that they differ unequivocally in localization and structure from the changes produced by smoke. The findings show that the two factors smoke and nitrosamin have different places of attack (see graph 66), and that they are therefore not able to influence each other in their effect on the same place. The conclusion could be drawn from these results that nitrosamin does not deserve the significance in smoke which individual authors have attributed to it (DRUCKREY and PREUSSMANN, 1962; KROLLER, 1967; MEURATH, 1969; MEURATH and Assoc. 1965; PALLER and KLUS, 1971; PREUSSMANN and EISENBRAND, 1972; RHOADES and JOHNSON, 1970, 1972; SERFONTAIN and MURTER, 1965). This question, much discussed today, should be elucidated through further experiments. The answering of this question is complicated by the fact, that the apparently in smoke most strongly represented dimethyl-nitrosamin (DMBA) shows a carcinogenic effect solely in the liver of the hamster but not in the lung. Even in the liver we could not find tumors more frequently in the animal groups which were smoke-treated with cigarettes containing a high additive of NaNO_3 . Since however, most other nitrosamins,

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suspected or discovered in smoke, are carcinogenic in the hamster, this result deserves a rather large significance.

The problems of the nitrosamins must once again be placed in the foreground in the comparison of the effect of individual smoke-qualities. Since several authors (DRUCK-REY and FREUSSMANN, 1962, RHOADES and JOHNSON, 1972) are of the opinion that the admixture of NaNO_3 favours a nitrosamin formation in the smoke, either an increased effect on the larynx should appear with a high NaNO_3 admixture, in the place where it also appears through smoke alone, or in those places where the changes are found with nitrosamin treatment. This however, was not the case with the nitrate admixture. The hypothesis of a nitrosamin effect in smoke has not found affirmation through the results of these experiments.

5. Besides the changes resulting from the smoke-treatment and cited as proof for the usefulness of the method, we plan to discuss in the following the remaining findings which we obtained. Chart I35 offers a good survey. Among the lung changes, we should mention rarely appearing adenomas which do not occur unequivocally more frequently with the smoke-treatment. A lung carcinoma occurred only in one animal, treated simultaneously with D₁BA and smoke. Also in the remaining tumors of the pharynx and the trachea, of the cavity of the nose and the oral cavity, a higher frequency appeared only in those animals which received D₁BA in addition.

Interesting is the papilloma, carcinoma and ulcer frequency in the stomach of the test animals. These changes apparently occur in a number of animals spontaneously. A difference in the frequency is not ascertainable between the smoke-exposed animals and the controls. An extreme rise in the papilloma frequency was recognizable solely in the animals of groups I and 2, treated with D₁BA. However, no difference exists between the two groups. This result permits the conclusion that a carcinogenic or cocarcinogenic effect after smoke-treatment does not occur in the stomach.

The testicle changes appearing in the hamster in the sense of a testicle atrophy are

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in parts, age-dependent, in parts, apparently caused through mechanical factors of the test arrangement. The comparative examinations of the testicle weights of smoke-exposed animals, animals treated with fresh air and untreated control animals show that the greatest loss in testicle weight is caused by the test arrangement, since fresh-air animals demonstrate a considerable reduction in the testicle weight already after a treatment of three months. The multiple transfers of the animals into the smoke tubes apparently produces a mechanical alteration and displacement of the testicles which are very sensitive to mechanical and thermic factors, as the literature survey shows. The somewhat stronger loss in testicle weight in the smoke-exposed animals could be expression of an additional influence. According to the entire findings, the hamster is not suitable for the evaluation of influences of smoke-components on the testicle tissue.

The frequently observed amyloidosis in the hamster, particularly the variously severe amyloidosis of the kidney to the point of the shrunken kidney shows no influence of the smoke-treatment. The amyloidosis is essentially age-dependent, as described already earlier by DONTENWILL and Assoc. (1960). In the severe amyloidosis, stage III, a weight increase of the heart is above all traceable in the untreated animals, which can be interpreted as a rise in blood-pressure through kidney shrinking. These changes however, do not lead to the pattern of an arteriosclerosis in the area of the aorta, the coronary arteries or the vessels of the leg.

The obtained heart changes, the changes on the valves as well as on the muscular system of the heart, are provable in smoke-exposed and not smoke-exposed animals. Also the findings on the vessels in the sense of an arteriitis (heart vessels and peripheral vessels) can be recognized in approximately the same frequency in the controls and the smoke-exposed animals. The present experiments do not show an etiologic influence of smoking on the formation of the described vessel processes.

The degeneration of the muscular systems of the skeleton and the heart which can be

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observed in the hamster are not influenced by the smoke-treatment or the treatment with carcinogens.

The frequency of the tumors of the suprarenal gland, which belong to the most frequent spontaneous tumors in the hamster, does not show an influence of smoke-components.

The in all groups approximately equally frequent inflammable changes, particularly in the form of phlegmonous, subcutaneous inflammations as well as inflammable infiltration in the area of the head starting from the mucous membrane of the mouth, are not in any correlation to the smoke-treatment.

In a comparative observation of the remaining spontaneous tumors, carcinomas and sarcomas, e.g. carcinomas of the urinary bladder, tumors of the suprarenal gland and the liver as well as tumors of the lymphatic and reticular tissue, an unequivocal increase is neither provable in the smoke-exposed animals nor in the animals treated with carcinogens. That does not refer to the tumors of the oral cavity, the pharynx, the esophagus and oesum after DLBA-treatment as well as the papillomas in the lower larynx and in the trachea after nitrosamin-treatment. In the animals treated with DLBA, the tumors of the lymphatic tissue appear earlier or, in the earlier deceased animals, are diagnosed earlier.

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V. Condensation

It was reported on a smoke-treatment experiment with cigarette-smoke, in parts, with simultaneous treatment with carcinogenic substances which embraced a total of 4.440 goldhamsters. 3.600 of these animals were treated over their entire lifetime, while the remaining 840 animals were smoke-treated up to 52 weeks.

The tests were processed and statistically evaluated with the aid of electronic data processing. In the test results were included survival period as well as body and organ weight development which were correlated, in parts, with the conducted treatment, respectively with the obtained morphological changes. Extensive patho-histological examinations of the organs and the tissue were carried out in order to seize all test-contingent changes.

Hematological as well as biochemical blood-tests were conducted on a large number of animals.

The present smoke-treatment experiment demonstrates that the developed method yields reproducible values in which a dose-effect correlation is provable. The method allows for a comparison of the effectiveness of the smoke from various cigarettes. The tests also enable an evaluation of combination treatments, e. g. smoke-treatment and treatment with carcinogens. A distinction was made in the obtained changes between:

I. Findings which were unequivocally produced by the smoke-treatment. Standing thereby in the foreground are larynx changes which we divided into 6 stages of severity. The appearance of larynx changes is dose-dependent. In a comparison between individual test groups distinct differences can be recognized. With respect to these changes it was possible to demonstrate unequivocally that certain test cigarettes (e. g. filter, cigarettes from a certain sheet or cigarettes with sodium-nitrate-additive) show an

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unequivocal reduction of the biological activity as measured on the larynx changes. This is particularly evident when the frequency of the most severe changes (stage 5 - pseudoepitheliomatous leukoplakia and stage 6 - carcinomas) is compared between the groups. The tests show furthermore that the appearance of the stages 2 - 6 is dependent on the length of the treatment and on the dose.

Upon combination of DMBA and smoke-treatment an over-additive effect becomes evident. While DMBA and smoke attack in the same localities, a simultaneous nitrosamin treatment demonstrated that smoke and DENA show different points of attack in the respiratory tract. The significance of these findings is under discussion.

The smoke-treatment leads to a dose-dependent shortening of the survival period and to body-weight decrease.

The smoke-treatment caused an increase in the erythrocytes and in the hemoglobin. This finding is an expression of the high smoke-dosing contingent upon the test arrangement.

2. Findings which are unequivocally strengthened through the smoke-treatment. The so-called "smoke-cells", also traceable in the controls, are with high significance more frequent in the smoke-exposed animals, while the increase of the so-called "adenomatoid lesions" is only weakly ascertained in the smoke-exposed animals. The changes in the respiratory tract, triggered by DMBA, are over-additively intensified through the smoke-treatment.

3. Findings, triggered or intensified through DMBA, are: the increase in the tumors of the oral cavity, the palate, the pharynx, the esophagus, the stomach, the trachea, the liver and the ovaries and the appearance of ovarian cysts.

4. Findings, triggered by the nitrosamin-treatment, are papillomas of the lower larynx which do not appear in the same manner in the smoke-exposed animals, as well as papillomas of the trachea.

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5. Findings which do not demonstrate any provable correlation to the treatment:

brain findings, tumors of the cavity of the nose, stomach ulcers (no dose-dependency), gastritis, lung emphysema, inflammations of the respiratory tract, generalized amyloidosis, testicle atrophy, vascular and heart diseases, muscular atrophy, formation of thrombi in heart and lung, cardiomyopathy, inflammable changes of the inguinal regions and the intestines, bile-duct cysts, bile-duct proliferation etc., Tumors of the skin, the connective and supportive tissue, the hematopoietic system and the suprarenal gland, hematological findings (except for erythrocytes and hemoglobin), biochemical findings.

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